1956

# Some observations on oral protozoa of man

Howard Edwin Runion
*University of the Pacific*

# SOME OBSERVATIONS ON ORAL PROTOZOA OF MAN

A Thesis

Presented to

the Faculty of the Department of Zoology

College of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Howard Edwin Runion

June 1956

TABLE OF CONTENTS

LIST OF CHARTS

CHART PAGE

CHART PAGE

# INTRODUCTION

## The Problem

This investigation is based upon the occurrence of oral protozoans among 25 periodontoclasial patients in Stockton, California. It is concerned, in part, with the correlation of infections with age, sex and oral health of the host and, in part, with culture techniques. The specific cultivation of Trichomonas tenax was explored in an effort to employ an improved experimental medium.

Trichomonas tenax and Endamoeba gingivalis are the only protozoans known to occur commonly in the human mouth. Distribution is cosmopolitan and there are no known endemic areas. Incidence of these two protozoans, among periodontoclasial patients, has not been reported since the study made by Jirovec, Bartos, Mezl and Novack (1942).

## History of Trichomonas tenax

Trichomonas tenax was described by O. F. Müller (1773) as a pear shaped organism. He named the flagellate Cercaria tenax, identifying it with a genus now known to represent a developmental stage in the life of certain trematode worms. Steinberg (1862) described these flagellates as members of

three species: Trichomonas elongata, T. caucata and T. flagellata. The morphological differences between the three alleged species were somewhat superficial, i.e., differences in shape and size. Subsequent investigators have not recognized these three alleged species. Goodey (1917) gave the organism the name Tetratrichomonas buccalis. Dobell (1939) demonstrated that the rules of priority necessitated the designation Trichomonas tenax.

History of Endamoeba gingivalis

Endamoeba gingivalis, the first amoeba to be taken from the human body, was discovered by Gros (1849) who made scrapings of tartar surrounding the base of teeth. He gave this organism the name Amoeba gingivalis. Grassi (1879) reported organisms found in the mouth which he called Amoeba dentalis. Prowazek (1904), unaware of Grassi's work, published accounts of his own observations and named the amoeba Entamoeba buccalis. The name E. buccalis was generally used previous to 1915 as the result of wide circulation of Prowazek's work. Brumpt (1913) called attention to the validity of the name given by Gros in 1849 and proposed that it be properly named Endamoeba gingivalis.

Barrett (1914) associated E. gingivalis with pyorrhoa alveolaris (periodontoclasia) and he treated his patients with Emetin Hydrochloride and other derivatives of ipecac, achiev-

ing some therapeutic results. Bass and Johns (1914) also claimed that E. gingivalis was the cause of pyorrhea alveolaris. In 1926 treatment to destroy the amoeba was abandoned finally following discoveries by numerous workers that E. gingivalis was not present in many pyorrhea cases and occasionally was found in healthy mouths.

Kirby (1928) found a heavy infection of an Endamoeba that closely resembled Endamoeba gingivalis in pyorrhea mouths of two Chimpanzees. Hegner (1929) reported the presence of an amoebae in mouths of a number of wild Philippine monkeys (Macacus philippinensis). Kofoid, Hinshaw and Johnstone (1929) discovered oral amoebae identical to Endamoeba gingivalis in several specimens of Macacus rhesus and M. cynomolgus. Kofoid and Johnstone (1930) again reported numerous monkeys from Asia as having Endamoeba gingivalis. Deschiens and Gourvii (1930) described E. gingivalis from mouths of monkeys. Hegner (1929) is of the opinion that protozoan parasites of monkeys are natural infections and not contaminations from man.

## METHODS AND MATERIALS

### Examination of the Periodontoclasial Patient

With generous cooperation from Dr. William H. Renwick D.D.S., Stockton, California, the writer was able to make contact with 25 periodontoclasial patients. Only patients

with periodontoclasia (pyorrhea alveolaris) of light, moderate, or heavy extents were examined. The degree of pyorrheal condition was determined by Dr. Renwick. Teeth were extracted from all those examined; each tooth was immediately placed in Boeck and Drbohlav's medium [modified by Howitt (1925)] and incubated at 37° C. Following extraction, a report was filled out by the doctor, supplying all necessary data concerning the patient. The cultures were transported from the dental office to the College of the Pacific laboratory inside the vest pocket to prevent cooling of the cultures.

Culture Medium for Endamoeba gingivalis

The culture medium used throughout the survey was Boeck and Drbohlav's media [modified by Howitt (1925)]. Boeck and Drbohlav (1924) described a new culture medium successful for the growth of Endamoeba histolytica. Howitt (1925) modified Boeck and Drbohlav's media for the cultivation of Endamoeba gingivalis. Trying various other types of media, Howitt found the modification of Boeck and Drbohlav's media produced the best results.

Culture Medium for Trichomonas tenax

Lynch (1915) was the first worker to successfully cultivate Trichomonas tenax. An acid bouillion culture media was

used, but this media would not maintain them indefinitely *in vitro*. Ohira and Nogucho (1917) reported considerable success with ascitic fluid and Ringer's solution. Lynch (1922) again reported further success in culturing *Trichomonas tenax* with varieties of protein solutions such as blood serum, ascitic fluid, ovarian cyst fluid and pleural fluid in physiological saline. Hogue (1926) maintained cultures of *T. tenax* in saline solution, containing sheep serum mixed with saliva. When covered with paraffin oil, single cultures lasted as long as thirty days without transplanting. Hinshaw (1927) found Boeck and Drbohlav's medium [modified by Howitt (1925)] quite satisfactory for cultivation of the oral flagellate.

## The Production of Boeck and Drbohlav's Medium [modified by Howitt (1925)]

The following three steps were used for making Boeck and Drbohlav's media [modified by Howitt (1925)]:

(1) Solid Egg Slant

Four fresh eggs were thoroughly washed, wiped and placed in ninety percent alcohol for fifteen minutes to sterilize the shell. The eggs were then cracked and mixed with 50ml of Locke solution in an electric food blendor until homogeneous. The material was tubed, slanted and autoclaved. It was essential that the autoclave be closed tightly and all valves shut before heating as the entraped air in the autoclave prevented the tem-

porature from rising above 200° F. at 15 pounds pressure. The culture slants were autoclaved from 190° F. to 200° F. at 15 pounds pressure for thirty minutes. The autoclave was rapidly exhausted, taking care that the temperature did not rise much above 200° F. at any time (Coagulation of egg slants in the Arnold sterilizer followed by autoclaving at normal temperatures is not recommended. Coagulation with the Arnold sterilizer alone will not assure sterile slants.).

(2) Locke Solution

Salt Solution

| | |
|---|---|
| NaCl | 360 grams |
| $CaCl_2$ | 8 grams |
| KCl | 16.8 grams |
| HOH | 2000ml |

Solution was sterilized thirty minutes at 15 pounds pressure.

Sodium Bicarbonate Solution

| | |
|---|---|
| $NaHCO_3$ | 8 grams |
| HOH | 100ml |

To prevent absorption of $CO_2$ from the air, the solution was not shaken; to sterilize, the solution might be filtered through a Berkofield filter. The writer autoclaved the solution with no apparent harm.

Sugar Solution

| | |
|---|---|
| Dextrose | 25.1 grams |
| HOH | 500ml |

Solution was sterilized in the Arnold sterilizer twenty minutes each day for three consecutive days.

The three basic solutions, when completed, were stored in the following amounts:

| | |
|---|---|
| Salt solution | 110ml units |
| Sodium Bicarbonate solution | 5ml units |
| Sugar solution | 10ml units |

(sealed in wax)

Locke solution was prepared by combining one unit each of the preceding solutions with 1,385ml of sterile distilled water. The writer found this method of preparing Locke solution relatively simple.

(3) Locke Albumen Solution

One egg was cleaned, wiped and placed in ninety percent alcohol fifteen minutes. The egg white was aseptically cracked into a sterile flask which contained glass beads with 500ml of sterile Locke solution. The albumen was blended completely into the solution. The Locke albumen solution was added aseptically over the Locke-egg-albumen slant.

The Production of Nelson's Medium (1947)

Nelson (1947) developed a new medium for cultivation of Endemoeba histolytica. Attempts to use this medium experimentally for cultivation of Trichomonas tenax proved quite successful. The procedure used by the writer for production of Nelson's medium was as follows: One hundred milliliters of ninety-five percent Ethyl alcohol were added slowly to one egg yoke which was completely free of albumen. To insure a homogeneous blend the mixture was stirred constantly as the alcohol was added. Dehydration was achieved by agitating the substance several times each day. After two days of dehydration, 10ml of the stock-alcohol-egg-extract were poured into a small flask; the alcohol was driven off by heating in a water bath. Twenty milliliters of melted two percent agar in buffered 0.5 percent saline were added to the preceding mixture. The mixture was then tubed and autoclaved at 15 pounds pressure for fifteen minutes. The egg particles separated from the hot agar during sterilization in the autoclave. Even distribution of the egg in a slant was obtained by shaking tubed material and slanting in ice water. The agar solidified rapidly, suspending egg particles evenly in the medium. The slants were covered with sterile buffered (pH 7.0), 0.5 percent saline solution. To provide supplementary nutrients for the organism 0.1mg of sterile rice starch can be added to the medium at time of innoculation.

Subculturing

Subculturing was accomplished by inserting a sterile, one milliliter pipette to the base of a slant where the organisms were most abundant. One milliliter of material was withdrawn from a stock culture and transferred to a prewarmed, 37° C., tube of fresh medium. The media were prewarmed just prior to inoculation to eliminate possible shock and death to the organism.

Cultures of Endamoeba gingivalis were transplanted every forty-eight hours while those of Trichomonas tenax were transplanted every three to five days.

Examination of Cultures

Determination of the presence of oral protozoans in cultured teeth from patients examined was limited to observations in vitro. Permanent slides were not used to prevent possible loss of specimens while fixing or staining slides.

The examination of cultures was divided into three steps: (1) removal of material from slant with a sterile wire loop, (2) transferal of material to a blood counting chamber which was maintained on a warming stage, 37° C., to permit sustained life in vitro, and (3) examination for Endamoeba gingivalis and Trichomonas tenax.

Examinations were made twice each day until protozoans were observed or for three days. If protozoans were not seen

at the end of three days, the patient was considered negative.

Staining of Fixed Mounts

In order to study the morphology of Endamoeba gingivalis and Trichomonas tenax permanent slides were prepared.

Fixing was successfully achieved by floating a coverslip with the smeared material containing the protozoans on the surface of hot Schaudinn's fixative, 55° C., for one or two minutes. Then the coverslip with the fixed material upon it was stained with Heidenhains Iron Hematoxylin and mounted.

RESULTS

Morphology and behavior of Endamoeba gingivalis

The only known form of Endamoeba gingivalis is the trophozoite; no cyst stage has been associated definitely with the amoeba.

Prowazek (1904), Chiavaro (1914), Smith, Middleton and Barrett (1914), Bass and Johns (1915), Smith and Barrett (1915) and Goodrich and Mosely (1916) have described the morphology of E. gingivalis. These and other observations dealt mainly with the animal in its living state and in the nature of nutrient inclusions. Detailed studies of the nucleus were made by Kofoid and Swezy (1924).

Most specimens measured by the writer ranged from 12 to 20 microns in diameter. The exterior surface of the amoeba appeared slightly waxy in the living state; the protoplasm was clear with the exception of granular material flowing in the direction of extending psuedopodia. The psuedopodia were broad, blunt, large and frequently thrust out with explosive rapidity, but were few in number. This amoeba often extended at once in several directions with one or more psuedopodia finally giving way to supply needed protoplasm to a larger, further extending limb. Also this amoeba frequently extended itself into a single unit resembling a sausage. Occasionally a sticky substance which caught nutrient particles and bacteria in its mass was observed trailing behind the amoeba. As this amoeba became cold, it appeared sluggish and usually rounded up.

With the use of Heidenhains Iron Hematoxylin, the nucleus, food vacuoles, and cytoplasmic inclusions were readily seen. The nuclei of leucocytes in the food vacuoles were frequently revealed in amoebae which were freshly removed from the oral cavity of patients and stained. However, bacteria was observed in the food vacuoles of the amoebae taken from the cultured stains and stained. The protein material provided by the culture was not observed in the food vacuoles and evidently did not stain with Heidenhains Iorn Hematoxylin. Chart I, page 14, illustrates the diagrematic structure of E. gingivalis.

Morphology and behavior of Trichomonas tenax

The first complete morphological description of Trichomonas tenax was made by Hinshaw (1926). He also gave accounts of mitotic division. Chart 2, figure 1, page 14, illustrates the diagramatic structure of T. tenax as prepared by the writer. No cyst forms have been definitely associated with T. tenax.

In the living state, the flagellate had a finely granular cytoplasm with an occasional vacuole. Locomotion was by the use of the four flagella rising from the anterior portion of the body, and an undulating membrane which rises from the anterior portion and extends downwards three fourths the length of the body. The animal was very active and it moved about rapidly. This was particularly noticable when placed in food masses of culture material. The flagellate attached the material with great zest and vigor, rarely stopping for a moment. If the organism was free of food particles and swimming in clear material, it slowed down for several minutes only to suddenly resume its previous activities. The pattern of motion was fairly uniform. The flagella appeared to work in groups of two: (1) two in rhythm with the undulating membrane, and (2) the other two functioning together or separately. The flagellum curled up and snapped like a whip with each new thrust. The T. tenax often swam in a wavy line so consistent in pattern that the waves were the same within a given time. Then the

protozoan abruptly changed this pattern to make loops, dips, whirls and twists. The axostyle appeared to act as a rudder and an anchor. The animal was observed frequently using the axostyle as an anchor on the undersurface of a slide while feeding or resting.

The writer saw no visible evidence of a cytostome, whereas Hinshaw (1926) reported an indefinite clear area, triangular in shape which was near the origin of the undulating membrane. He suggested this area was probably the cytostome.

Trichomonas tenax experienced autotomy when faced with an unfavorable change in environment (Chart 2, figure 2). The transferal of this flagellate from the mouth to culture media prompted autotomy. Often a lapse of forty-eight hours was necessary before T. tenax was readily observed in vitro. A number of residual bodies resulting from autotomy were frequently seen in fresh cultures from patients.

Incidence of Endamoeba gingivalis and Trichomonas tenax: observed by Previous writers

Following a report by Barrett (1914) that Endamoeba gingivalis was the possible cause of pyorrhea, surveys were made to determine prevelence of E. gingivalis and Trichomonas tenax. The results of these surveys were compiled by the writer to illustrate incidence of infection for Endamoeba gingivalis (Chart 3, page 16) and Trichomonas tenax (Chart 4, page 17).

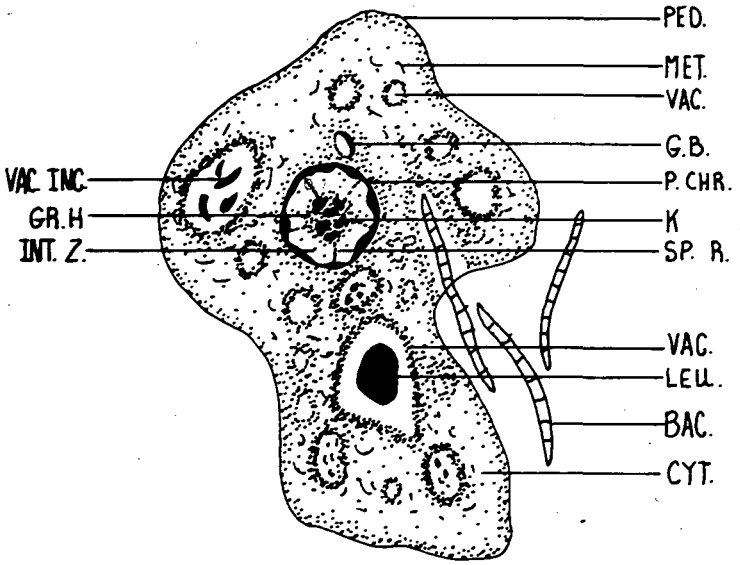


CHART 1

DIAGRAMMATIC FIGURE OF ENDAMOEBA GINGIVALIS (GROS)

KEY

BAC = BACTERIA
CYT. = CYTOPLASM
G. B = GOLGI BODY
GR. H = GRANULAR BODY
INT. Z = INTERMEDIATE ZONE
K = KARYOSOME
LEU. = LEUCOCYTE
MET. = MITOCHONDRIA
P. CHR = PERIPHERAL CHROMATIN
PED. = PELLICLE
SPR. = SPOKE RADII
VAC = VACUOLE
VAC. INC. = VACUOLE INCLUSION

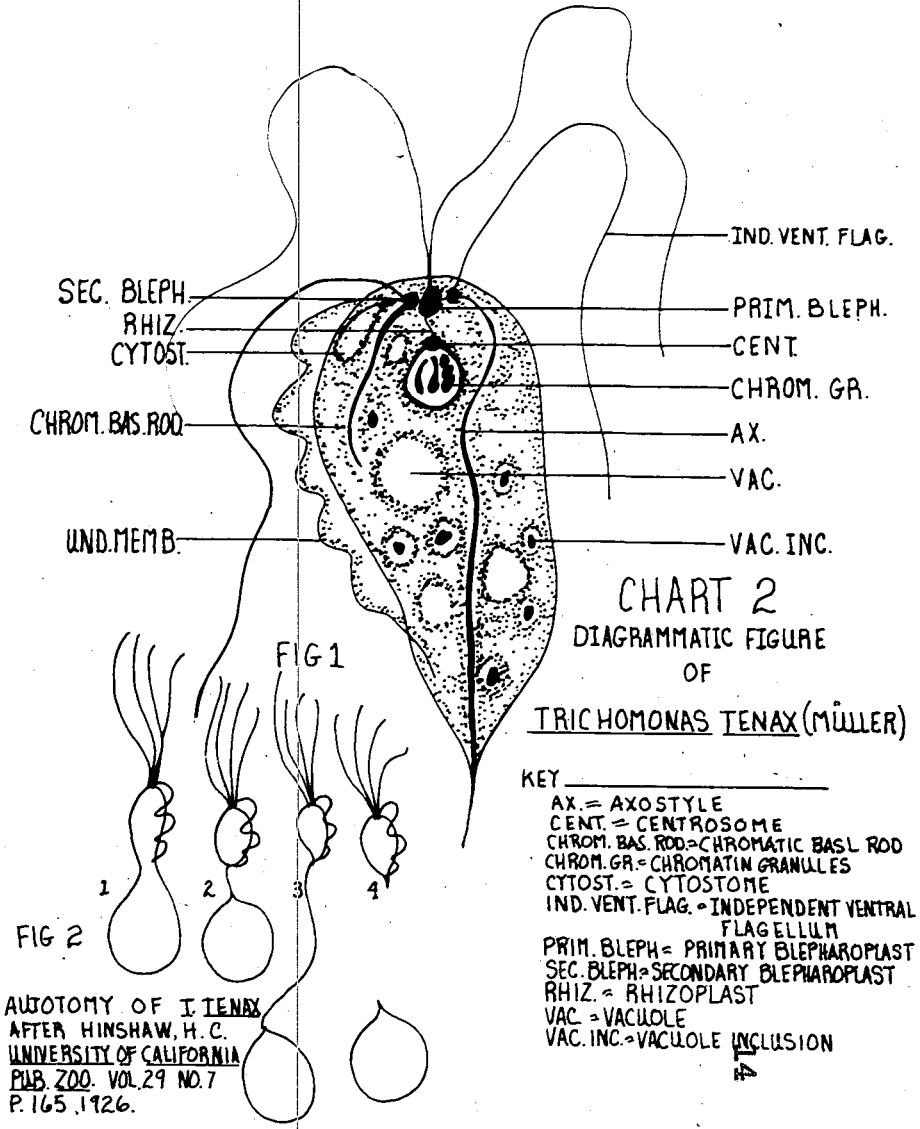


FIG 1




FIG 2

AUTOTOMY OF T. TENAX
AFTER HINSHAW, H. C.
UNIVERSITY OF CALIFORNIA
PUB. ZOO. VOL. 29 NO. 7
P. 165, 1926.

CHART 2

DIAGRAMMATIC FIGURE OF TRICHOMONAS TENAX (MÜLLER)

KEY

AX. = AXOSTYLE
CENT. = CENTROSOME
CHROM. BAS. ROD = CHROMATIC BASL ROD
CHROM. GR. = CHROMATIN GRANULES
CYTOST. = CYTOSTOME
IND. VENT. FLAG. = INDEPENDENT VENTRAL FLAGELLUM
PRIM. BLEPH = PRIMARY BLEPHAROPLAST
SEC. BLEPH = SECONDARY BLEPHAROPLAST
RHIZ. = RHIZOPLAST
VAC = VACUOLE
VAC. INC. = VACUOLE INCLUSION

The results of Jirovec, Bartos, Mezl and Novack's (1942) attempt to discover the relationship between the infection of Endamoeba gingivalis and Trichomonas tenax and the condition of the mouth, age of the individual and whether both protozoans were pathogenic were also compiled by the writer (Chart 5, page 18). Of 1,006 subjects examined in two European cities those with pyorrhea were found to have the highest percent of protozoan infestation. They reported the degree of infection was dependent upon: (1) age of the individual (Abdulabekow (1934) found children as young as thirty-one months infected with Endamoeba gingivalis.), and (2) magnitude of pyorrheal infection (Abulabekow (1934) found that men, women, and infants without teeth were not infected with oral protozoans.). Jirovec, Bartox, Mezl and Novack concluded that the pathogenic influence of either E. gingivalis or Trichomonas tenax was highly improbable. Treatment of the oral cavity and the oral hygenic measures which were taken were found ineffective in suppression of these protozoans.

Incidence of Endamoeba gingivalis and Trichomonas tenax observed by the writer

Twenty-five periodontoclasial cases (twelve female, thirteen male) were examined by the writer to determine the occurrence of oral protozoans. The incidence of protozoan infestation for the 25 persons examined (Chart 6, page 20) was sixty percent; twenty percent were infected with both Endamoeba gingi-

CHART 3

SURVEYS FOR ENDAMOEBA GINGIVALIS

| Number Examined | Percent Infected | Geographical area | Remarks | Authors |
|---|---|---|---|---|
| 201 | 95.5 | U.S.A. | Pyorrhoea cases | Fisher (1927) |
| 37 | 90.0 | U.S.A. | Pyorrhoea cases | Bass & Johns (1914) |
| ? | 31.0 | Japan | Pyorrhoea cases | Hiraoka (1936) |
| 1000 | 42.0 | Europe | All age groups | Abdulabekow (1934) |
| 1150 | 31.9 | Europe | | Drobhlav (1932) |

CHART 4

SURVEYS FOR TRICHOMONAS TENAX

| Number Examined | Percent Infected | Geographical area | Remarks | Authors |
|---|---|---|---|---|
| 500 | 95.5 | U.S.A. | Advanced pyorrhea | Hinshaw (1926) |
| 88 | 53.4 | Germany | Adults | Westphal (1936) |
| 50 | 52.0 | Malaya | Coolies | Jeeps (1923) |
| 186 | 30.6 | U.S.A. | Diseased mouths of prisoners | Hinshaw (1926) |
| 209 | 24.4 | U.S.A. | Whites, dental clinic | Beatman (1933) |
| 32 | 21.9 | U.S.A. | Dental clinic | Hogue (1926) |
| 141 | 18.4 | U.S.A. | Negro dental clinic | Beatman (1933) |
| 200 | 16.5 | U.S.A. | Unselected obstet. patients | Bland and Rukoff (1937) |
| 300 | 10.0 | England | Most with advanced Pyorrhea | Drew and Griffin (1917) |
| 72 | 7.0 | Germany | Women | Wagner and Hees (1937) |
| 50 | 4.0 | Germany | ----- | Back and Kiefer (1923) |

CHART 5

JIROVEC, BARTOX, MEZL AND NOVACK'S (1942) SURVEY

| Group Examined | Oral Protozoan | Prague | | Zlin | |
|---|---|---|---|---|---|
| | | Female | Male | Female | Male |
| General prevelence | T.T. | 18.8 | 20.0 | 16.4 | 10.3 |
| | E.G. | 7.0 | 1.0 | 3.0 | 5.8 |
| Middle aged - teeth intact | T.T. | 3.6 | | 12.5 | |
| | E.G. | 8.0 | | 4.2 | |
| Patients with caries, holes, crowns and bridges. | T.T. | 22.0 | | 20.2 | |
| | E.G. | 46.0 | | 5.4 | |
| Patients with heavy caries, heavy repairs and prostheses | T.T. | 21.0 | | 54.0 | |
| | E.G. | 50.0 | | 3.8 | |
| People with heavy Pyorrhea | T.T. | 32.0 | | 38.0 | |
| | E.G. | 60.0 | | 14.0 | |

NOTE: Figures are to be read as percents.

T.T. Trichomonas tenax E.G. Endamoeba gingivalis

valis and Trichomonas tenax. Twenty-four percent were infected with Endamoeba gingivalis and 16.0 percent with Trichomonas tenax. Sixty-nine and two tenths percent of the males examined were found with protozoan infestations; 7.8 percent were infested with both organisms; 46.1 percent harbored only Endamoeba gingivalis and 14.6 percent had Trichomonas tenax. Females examined showed 50.0 percent with a protozoan infestation; 33.3 percent possessed both organisms. While no Endamoeba gingivalis was found in the females, 16.6 percent possessed Trichomonas tenax.

The relationships between the degree of pyorrhea, sex, age and protozoan infestation were compiled by the writer (Charts 7 through 12, pages 20 through 22 and Chart 13, page 25).

Experimental Cultivation of Trichomonas tenax:

As Trichomonas tenax was found to be a very hearty organism and consequently easy to maintain, it was used exclusively for all experimental cultivations of this investigation.

The expected length life of T. tenax in culture was the first problem to be resolved because the organism died in culture as a result of bacteriostasis. The byproducts of bacterial metabolism which resulted from an overgrowth of bacteria produced a "poisoning" of the flagellate. Infrequent subculturing indangered the life of the organism, whereas subculturing at an optimum time removed it from an unhealthy environment.

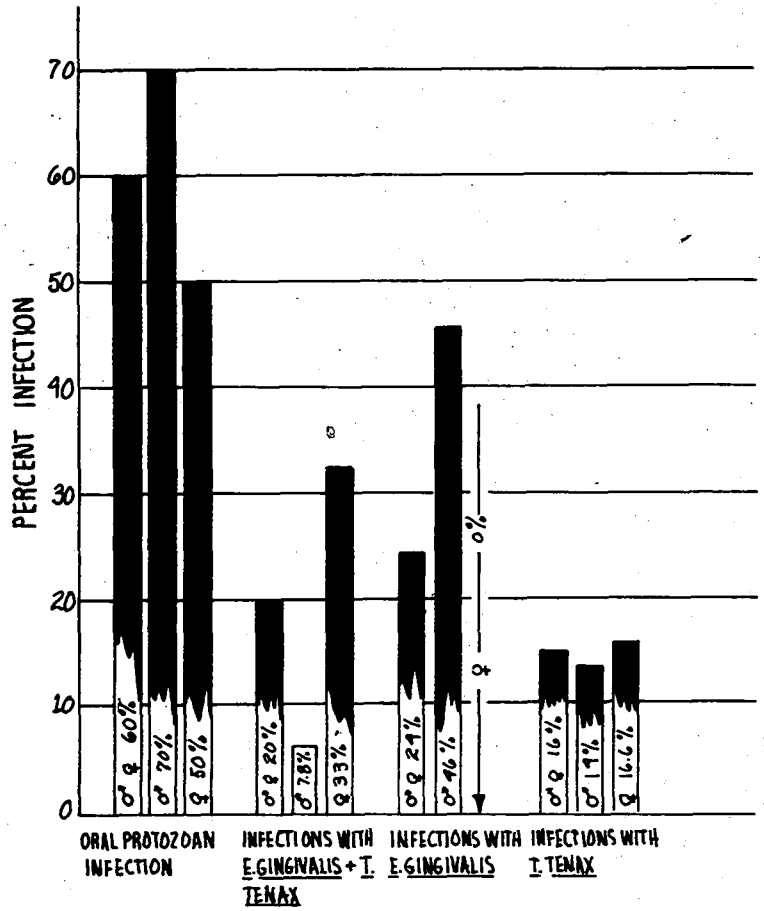


CHART 6

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 25 PERIODONTOCLASIAL PATIENTS FROM STOCKTON, CALIFORNIA.

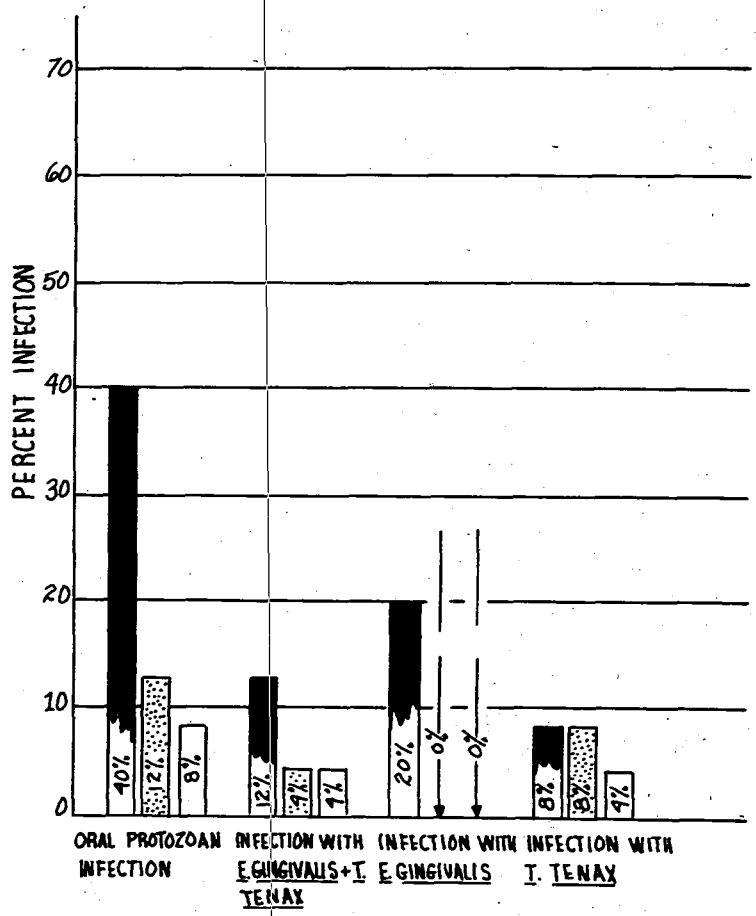


CHART 7

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 25 PERIODONTOCLASIAL PATIENTS WITH HEAVY, MODERATE, AND LIGHT PYORRHEA INFECTIONS FROM STOCKTON, CALIFORNIA.

LEGEND
HEAVY PYORRHEA
MODERATE PYORRHEA
LIGHT PYORRHEA

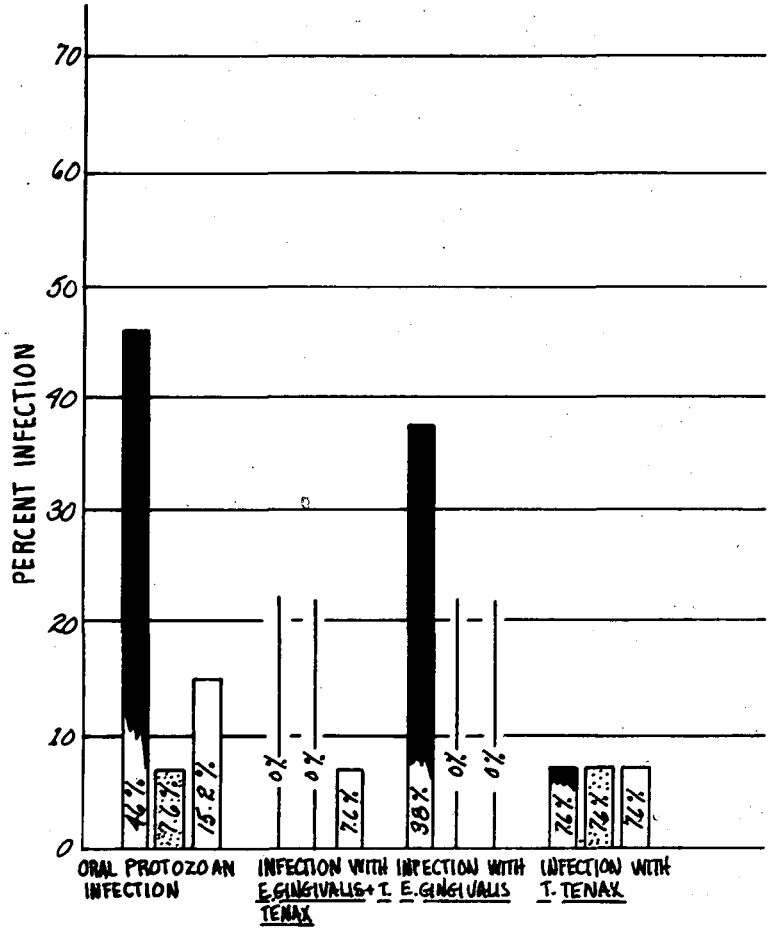


CHART 8

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 13 MALE PERIODONTOCLASIAL PATIENTS WITH HEAVY, MODERATE AND LIGHT PYORRHEA INFECTIONS FROM STOCKTON, CALIFORNIA.

LEGEND
HEAVY PYORRHEA
MODERATE PYORRHEA
LIGHT PYORRHEA

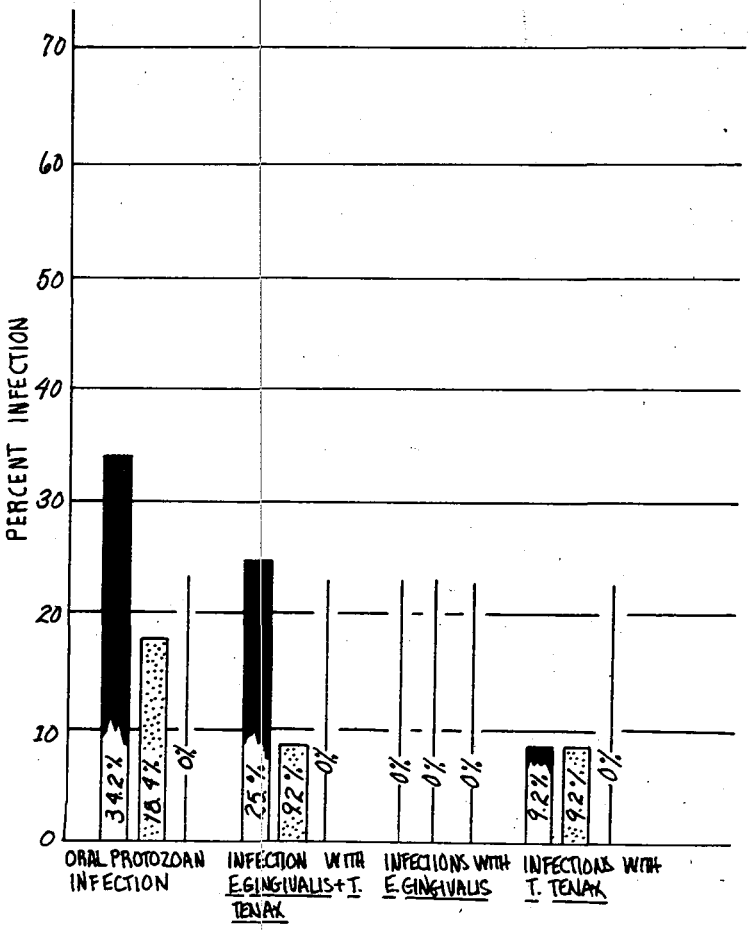


CHART 9

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 12 FEMALE PERIODONTOCLASIAL PATIENTS WITH HEAVY, MODERATE AND LIGHT PYORRHEA FROM STOCKTON, CALIFORNIA.

LEGEND
HEAVY PYORRHEA
MODERATE PYORRHEA
LIGHT PYORRHEA

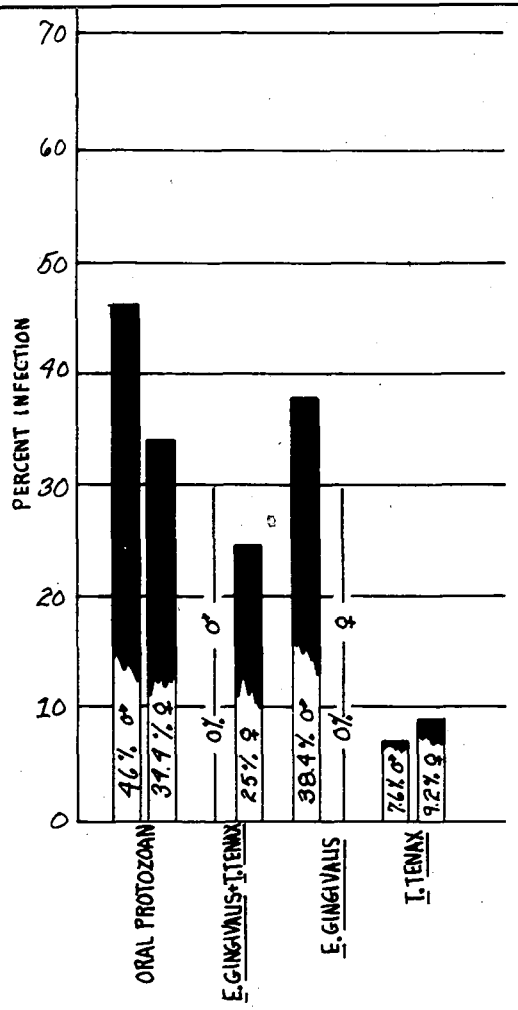


CHART 10

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 25 MALE AND FEMALE PERIODONTOCLASIAL PATIENTS WITH HEAVY PYORRHEA FROM STOCKTON, CALIFORNIA.

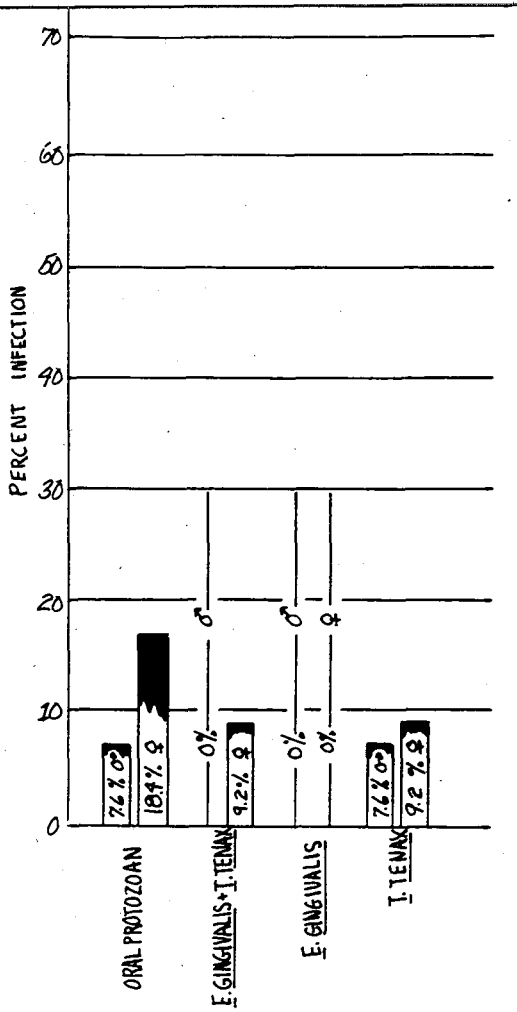


CHART 11

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 25 MALE AND FEMALE PERIODONTOCLASIAL PATIENTS WITH MODERATE PYORRHEA FROM STOCKTON, CALIFORNIA.

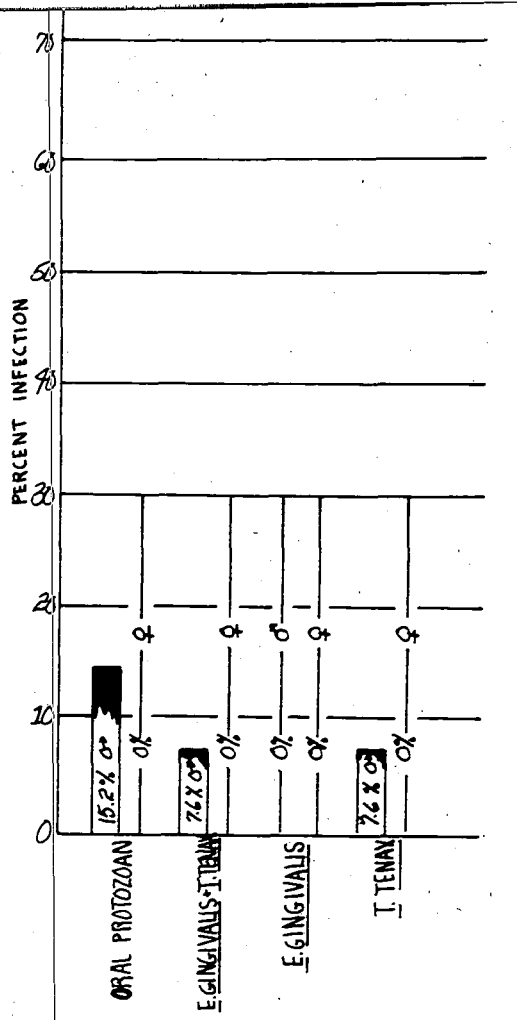


CHART 12

THE INCIDENCE OF TRICHOMONAS TENAX (MÜLLER) AND ENDAMOEBA GINGIVALIS (GROS) AMONG 25 MALE AND FEMALE PERIODONTOCLASIAL PATIENTS WITH LIGHT PYORRHEA FROM STOCKTON, CALIFORNIA.

Twenty-three cultures were examined to resolve the minimum, maximum and average length of life of T. tenax in culture Boeck and Drobahlav's Locke-egg-albumen (L.E.A.) medium [modified by Howitt, (1925)] without transplantation. Each culture was inoculated with one milliliter of supernatant material and incubated at 37° C. Following inoculation the life span was: (1) three days minimum, (2) twenty-six days maximum and (3) the average length of life for all cultures was 8.1 days (Chart 14, page 26).

Optimum growth of T. tenax was accomplished in a slightly alkaline media; however, following inoculation increased bacterial growth produced an acidic condition. The pH of five fresh cultures was recorded at 7.2, but forty-eight hours later the pH ranged 5.9, 6.0, 6.05, 6.35 and 6.5. Although no attempt was made to determine the chemical composition of the culture media, the characteristic odor of butyric acid was evident twenty-four to forty-eight hours following innoculation. The supernatant, composed of Locke solution and albumen, always turned a milky white and a layer of this material deposited on the surface of the slant. T. tenax was quite abundant in this deposited material.

The second problem was to determine the length of life of T. tenax in Boeck and Drobahlav's medium [modified by Howitt (1925)] when subjected to varying conditions. The length of life as determined when the flagellate was subjected to these

various conditions are illustrated in Chart 15, page 26.

The effect of penicillin upon the bacteria and T. tenax was the third proposition explored in the writer's effort to find an improved experimental medium for cultivation of T. tenax. As illustrated in Chart 15, page 26, the bacteria were reduced. A sedative effect was noted in the generally active trichomonad. It contracted into a rounded ball similar to the residual bodies of autotomy and while the undulating membrane moved constantly, the flagellate remained relatively quiet. Also the odor of butyric acid was not noted when penicillin was injected into the medium.

The fourth step explored in order to find an improved experimental medium for cultivation of T. tenax was experimental use of Nelson's medium (1947). No attempts were made to cultivate Endamoeba gingivalis as cultivation of Trichomonas tenax was the writer's prime focal point for an improved medium. Chart 16, page 25, illustrates the results observed by the writer when using Nelson's medium (1947) for cultivation of T. tenax. Penicillin was also used with this medium but no definite reactions were observed.

## DISCUSSION

The survey presented by this writer must be considered

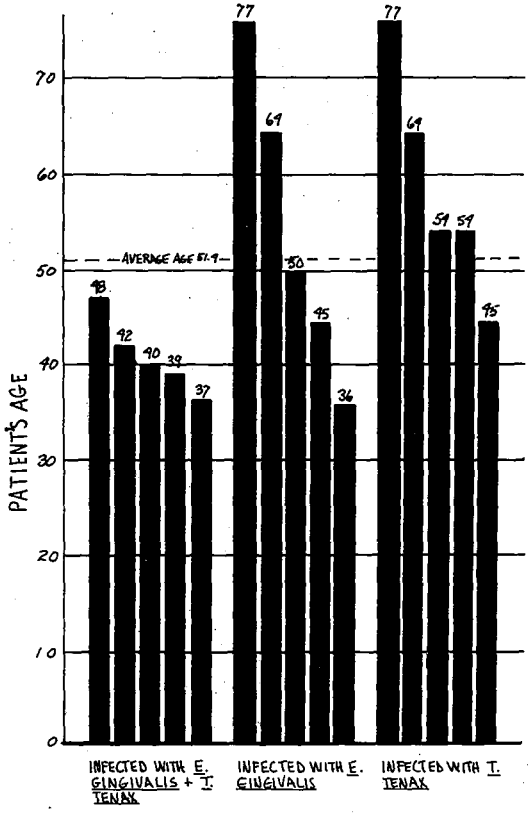


CHART 13

THE INCIDENCE OF ENDAMOEBA GINGIVALIS (GROS) AND TRICHOMONAS TENAX (MÜLLER) INFECTION AS TO AGE AND PROTOZOAN PRESENT.

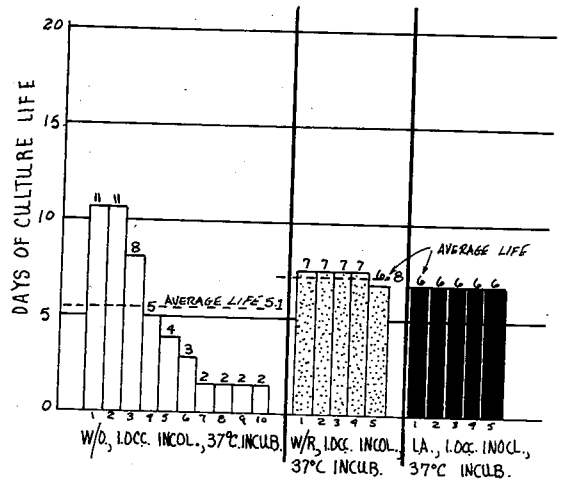


CHART 16

THE LIFE SPAN OF TRICHOMONAS TENAX (MÜLLER) UNDER VARYING CONDITIONS IN NELSON'S MEDIUM (1947).

TERMS KEY
W/O = WITHOUT RICE.
W/R = WITH RICE.

INCOL. = INNOCULATED TO EACH CULTURE.
INCUB. = INCUBATOR.
LA = LOCKE ALBUMEN SUPERNATANT OVER NELSON'S SLANT.

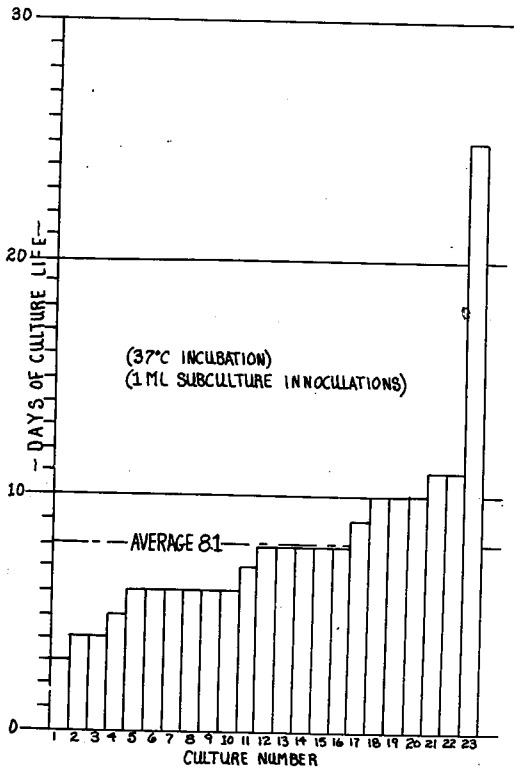


CHART 14

LIFE SPAN FOR TRICHOMONAS TENAX (MÜLLER) IN BOECK DRBOHLAV'S MEDIUM (MODIFIED BY HOWITT 1925).

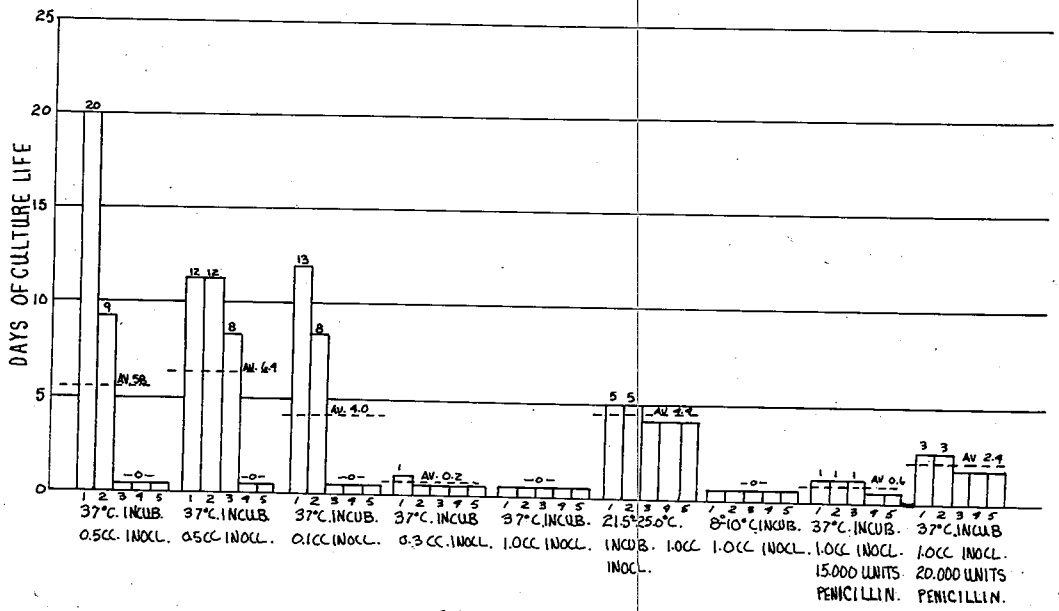


CHART 15

LIFE SPAN FOR TRICHOMONAS TENAX (MÜLLER) UNDER VARYING CONDITIONS IN BOECK AND DRBOHLAV'S MEDIA (MODIFIED BY HOWITT 1925).

TERMS KEY
AV. = AVERAGE DAYS GROWTH
INCUB. = INCUBATOR
INCOL. = INNOCULATED TO EACH CULTURE

indicative rather than conclusive. The findings which were gathered from only 25 individuals are not sufficient to uphold anything other than tentative conclusions. However, a review of literature provides results which, in many instances, definitely coincide with the writer's findings.

Correlation of Infections with Age, Sex and Oral Health of Host

Sixty percent of the twenty-five periodontoclasial patients examined by the writer were found to have an oral protozoan infestation (Chart 6, page 20). While there appeared to be a parallel between the incidence of oral protozoan infection and degree of pyorrhea (Chart 7, page 20), there did not appear to be a definite relationship between an individual's age, sex, degree of pyorrhea and/or the presence of Endamoeba gingivalis and/or Trichomonas tenax (Chart 8 through 13, pages 21, 22 and 25). Instead the presence of one and/or both species of the oral protozoans seemed to be the effect of an original infection by a chance contact. The result was an infestation by the specie and/or species from the mouth of the vector. The ability of the specie and/or species to live in the mouth of the host depended upon the condition of that individual's oral cavity, i.e., its ability to support the contracted organism and/or organisms (An individual with heavy pyorrhea, but free from a protozoan infection will be extremely vulnerable to contraction and maintenance of oral protozoa. Conversely,

an individual with a healthy mouth probably will not support growth of the oral protozoa even when once contracted.).

Culture Techniques

The techniques of cultivation and the environmental factors effecting growth in culture were limited to Trichomonas tenax. No difficulties were ever found by the writer in maintaining this flagellate's strains over an indefinite period of time.

The principle medium used by the writer was Boeck and Drbohlav's media [modified by Howitt (1925)]. This medium was found to be very satisfactory for producing healthy strains of both Endamoeba gingivalis and Trichomonas tenax, but Endamoeba gingivalis died within forty-eight hours after inoculation if Trichomonas tenax was present. Therefore, each protozoan, if to be grown successfully, had to be cultivated separately.

Although this medium was satisfactory and produced healthy strains of Trichomonas tenax, there were four undesirable features: (1) the length of time required for its preparation, (2) the inability by the writer to obtain a sterile medium (note following paragraph), (3) the difficulty of cleaning the exhausted cultures (The egg was a difficult and time consumming item to remove from the test tube.) and (4) the offensive odor produced by bacteria supported in this type medium.

The bacteria evidently gained admission into this culture medium through the Locke-albumen supernatant material which coagulated when autoclaved, although, Boeck and Drbohlav's slant media was successfully sterilized when autoclaved. While a sterile culture medium was not successfully produced by the writer, precaution was taken to inhibit growth of bacteria (medium was stored at 4° C.). Even with bacteria present in the culture medium, there was no apparent harm effected upon the Trichomonas tenax for they grew well. However, the possibility that growth could have been better without the presence of bacteria was not disregarded.

The presence of bacteria in this medium might be necessary. Efforts were made by the writer to reduce the amount of material required for each innoculation in hope that fewer stock cultures were needed (An undesirable feature of this medium was the length of time required for preparation.). Results were negative. One milliliter of material was needed for cultivation of T. tenax because apparently it was necessary to have an ample supply of digested material, bacteria and protozoa, to insure growth and reproduction in the new culture. It was observed by the writer that possibly certain components of this medium which were necessary for nutrition of the protozoa had to be broken down. The bacteria was necessary for this "breaking-down" process.

The second medium used by the writer was Nelson's

medium (1947). While the Trichomonas tenax life span was from three to twenty-six days in Boeck and Drbohlav's media [modified by Howitt (1925)], Nelson's medium did not support any individual culture longer than eleven days (Charts 14 and 16, pages 26 and 25). However, the total results were actually more consistant. Trichomonas tenax grew rapidly and profusely in this medium. Death of all the organisms in this culture medium occurred at about the same time.

The addition of 0.1mg sterile rice starch at time of innoculation also produced consistent results with Nelson's medium (1947). The flagellate population remained heavy and vigorous and the use of the sterile rice starch sustained life one or two days longer.

This medium was satisfactory and produced healthy strains of T. tenax and the four undesirable features of Boeck and Drbohlav's media were absent. Instead Nelson's medium (1947) was: (1) easy to prepare (It required less time to prepare and the constant attention was not required.), (2) less prone to produce bacteria (This supernatant did not support bacteria as did Boeck and Drbohlav's supernatant.), (3) easy to clean exhausted cultures (Agar melted and floated away when washed.) and (4) it was without the offensive odor produced by bacteria which were not as profuse in this medium as in Boeck and Drbohlav's medium [modified by Howitt (1925)]. Therefore, the writer, with the combination of factors mentioned, be-

lieved Nelson's medium (1947) to be the superior of the two media used for the culture of T. tenax.

The use of penicillin to destroy the bacteria resulting in bacteria free cultures was only partially successful. Various trials were conducted with penicillin in amounts ra ranging from 5,000 to 20,000 units. Only cultures with either 15,00 or 20,000 units of penicillin supported life. The answer to this problem might be that the death of certain beneficial bacteria occurred with the addition of small amounts of penicillin while the bacteria responsible for the production of toxic byproducts were not destroyed until the 15,000 to 20,000 unit level was reached. If proper substances were provided for protozoan metabolism other than bacteria, success could be realized. The fact that some success was achieved for T. tenax indicated that further efforts might accomplish the desired result.

## SUMMARY

A study has been made of twenty-five pyorrheal patients, in Stockton, California, to determine the incidence of Trichomonas tenax amd/or Endamoeba gingivalis. The relationship between sex, age, and degree of Pyorrhea and presence of Trichomonas tenax amd/or Endamoeba gingivalis is given.

Boeck and Drbohlav's medium modified by Howitt(1925) and Nelson's medium (1947) were employed in cultivation of Trichomonas tenax under varying conditions. An evaluation of both media is made.

## LITERATURE CITED


Abdulabekow, Hamind.
1934. Uber die Verbreitung der Entamoeba gingivalis (Gros) 1849 in Aserbjdschan. Vorlaufige Mitteil. Arch. Schiffs. u. Tropen-Hyg., 38(4):174-177.

Bach, F. W., and Kiefer, K. H.
1933. Ueber die Verbreitung parasitischer Darmprotozoenarten des Menschen innerhalb Deutschlands. Arch. Schiffs. u. Tropenhyg., 27:145-169.

Barrett, M. T.
1914. Protozoa of the mouth in relation to pyorrhoea alveolaris. Dent. Cosmos., 56:948-953.

Bass, C. C., and Johns, F. M.
1914. Unpublished report to the Orleans Parish Medical Society, September.

Bass, C. C. and Johns, F. M.
1915. Alveolodental pyorrhea (Philadelphia, Saunders), 167 pp., 1 pl., 42 figs. in text.

Beatman, L. H.
1933. Studies in Trichomonas buccalis. J. Dent. Res., 13:339.

Bland, P. B., and Rakoff, A. E.
1937. The incidence of trichomonads in the vagina, mouths and rectum. Evidence that vaginal trichomonads do not originate in the mouth or intestine. J. Am Med. Assoc., 108:2013-2016.

Boeck, W. C., and Drbohlav, J.
1924. The cultivation of a pathogenic amoeba (Endamoeba histolytica) from a case of relapsed amoebic dysentery. MS. read before the American Society of Tropical Medicine, Chicago, June 9-10, 1924. Cited in "Minutes," Am. Jour. Trop. Med., 4:440.

Brumpt, E.
1913. Precis de parasitologie; ed. 2, Paris; Masson et Cie.

Chiavaro, A.
1914. Researches upon Entamoeba buccalis. Dent. Rev., 28:1122-1134, 10 figs. on one plate.

Dobell, C.
1939. The common flagellate of the human mouth, Trichomonas tenax (OFM), its discovery and its nomenclature. Parasitology, 31:138-146.

Deschiens, R., and Gourvil, E.
1930. Les entaibes de la bouche des singes. Bull. Soc. Path. Exotique, 23(7):711-714, 1 fig.

Drbohlav, Jaroslav.
1930. Signification de l'Entamoeba gingivalis in Stomatologie. Trav. Inst. Hyg. Publ. Etat Tchecoslovaque, 3(1):12-16, 1 pl.

Drew, A. H., and Griffen, V. D.
1917. The parasitology of pyorrhea alveolaris. J. Roy. Micr. Soc (Part 2):185-197.

Fisher, John H.
1927. Pyorrhea alveolaris: The rile of certain microorganisms found in the lesions. Amer. Jour. Path., 3(2):169-178, 1 pl.

Goodey, T.
1917. Note on the trichomonad flagellate Tetratrichomonas buccalis, N. sp. Parasitology, 9:554-557.

Goodrich, H. T., and Moseley, M.
1916. On certain parasites of the mouth in cases of pyorrhea. Jour. Roy. Micr. Soc., 1916, 513-527, pls. 12-17.

Grassi, B.
1879. Dei protozoi parassittie specialmente de quelli che sono nell uomo. Gass. med. ital., Lombardy, 39:445-448.

Gros, C.
1849. Fragments d'Helminthologie et de physiologie microscopique. Bull. Soc. Imp. de Nat. de Moscow, 22: 540-573.

Hegner, R.
1929. The protozoa of wild monkeys. Sci., 70:539-540.

Hinshaw, H. C.
1926. On the morphology and mitosis of Trichomonas buccalis (Goodey) Kofoid. Univ. Calif. Pub. Zool., 29:159-174.

Hinshaw, H. Corwin.
1927. Cultivation of Trichomonas buccalis, a protozoan of the human mouth. Univ. Calif. Publ. Zool., 31(4): 31-49.

Hiraoka, S. H.
1936. Experimentalle Studien uber Entamoeba buccalis. 1-7 (in Jap. with Ger. summ. p29) Fukuoka Acta Med. (Fukuoka-Ikwadaigaku-Zasshi) 29(5):607-703, 7 pl.

Hogue, M. J.
1926. Studies on Trichomonas buccalis. Am. Jour. Trop. Med., 6:75-88, 1 pl.

* Howitt, Beatrice Fay
1925. The cultivation of Endamoeba gingivalis (Gros). Univ. Calif. Publ. Zool., 28:65-126.

* Jepps, M.
1925. Protozoa of the human mouth in Malaya, Entamoeba gingivalis and Trichomonas sp. Parasitology, 15:343-347.

Jirovec, O., Frantisek Bartos, Zdenek Mezl, and Vojteck Novack.
1949. Oral protozoa in man. Arch. Protistenk, 96(1):31-38.

Kirby, H. Jr.
1929. Notes on some parasites from chimpanzees. Proc. Soc. Exp. Biol. Med., 25:698-700.

* Kofoid, C. A., and Swezy, O.
1924. The cytology of Endamoeba gingivalis (Gros) Brumpt, with special reference to the determination of the amoeba in bone marrow in arthritis deformas of Ely's second type. Univ. Calif. Publ. Zool., 26:165-198, pls. 14-16, 1 fig. in text.

* Kofoid, Charles A., Corwin Hinshaw, and Herbert G. Johnstone.
1929. Animal parasites of the mouth and their relation to dental diseases. Jour. Amer. Dental Assoc., (Aug): 20, 5 fig.

* Kofoid, C. A., and Johnstone, H. G.
1930. The oral amoeba of monkeys. Univ. Calif. Publ. Zool., 33(13):379, 2 pls.

* Lynch, K. M.
1915. Trichomoniasis of the vagina and the mouth, cultivation of the causative organism and experimental infection. Am. Jour. Trop. Dis. and Prevent. Med., 2:827-34.

* Lynch, K. M.
1922. Cultivation of Trichomonas and the differentiation flagellates. Jour. Am. Med. Assoc., 79:1130-33, 1 fig. in text.

Müller, O. F.
1773. Vermium terrestrium et fluviatilium seu animalium infusiorium, helminthocorum et testaceorum, non marinorum, succincta historia (Hafniae et Lipsiae, Heineck et Faber), 1(1):30-135; 1(2):72-88; 2:214.

Nelson, E. Clifford.
1947. Alcoholic extract medium for the diagnosis and cultivation of Endamoeba histolytica. Amer. Jour. Trop. Med. 27(5):545-552.

Ohira, T., and Noguchi, H.
1917. The cultivation of trichomonas from the human mouth (Tetratrichomonas hominis). Jour. Exper. Med., 25:341-47, pls. 34-37.

Prowazek, S. von.
1904. Entamoeba buccalis N. sp., Vorläufige Mitteilungen, Arb. a. d. K. Gesundhtsamte, 21:42-44.

Smith, A. J., Middleton, W. S., and Barrett, M. T.
1914: The tonsils as a habitat of oral endamebiasis. Jour. Am. Med. Ass., 63:1746-1749.

Smith, A. J., and Barrett, M. T.
1915. The parasite of oral and amebiasis, Endamoeba gingivalis (Gros). Jour. Parasit., 1:159-174.

Steinberg, S.
1862. Investigation of the soft white matter which accumulates on the teeth and between them. Souvemennaya Med., Kiev, 20, 24:377, 452.

Wagner, O., and Hees, E.
1937. 156 positive trichomonas blutbefunde bei Mensch und Tier. Zentr. Bakt. Parasitol. u. Infekt. Abt. I. Orig., 138:275-290.

Westphal, A.
1936. Zur Morphologie, Biologie und Infektionsfahigkeit de viergeisseligen Trichomonasarten des Menschen. Zentr. Bakt., Parasitol. u. Infekt. Abt. I. Orig. 127:363-376.